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Pacific Northwest
NATIONAL LABORATORY

Biological System Characterization to Address Biofouling of the 200 West Pump-and-Treat Injection Wells

September 2018

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U.S. DEPARTMENT OF ENERGY

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Richland, Washington 99352

Executive Summary

Contaminated groundwater in the 200W Area of the Hanford Site is being treated *ex situ* by the 200W Pump-and-Treat (P&T) Facility and the subsurface plume is under hydraulic containment. The 200W P&T Facility removes several contaminants of concern from groundwater, as well as nitrate. Facility capacity is constrained by biofouling of injection wells, which limits the ability to efficiently inject treated effluent water in to the subsurface.

In a previous report,[1] Thomle et al. provided a comprehensive engineering assessment of biofouling at the 200W P&T Facility. In that report, both operational changes and facility modifications were recommended to help mitigate the symptoms and consequences of biofouling by expanding treatment processes in the plant. The 200W P&T Facility's fluidized bed reactor (FBR) is a biological treatment system that has been identified as a principal source of biofouling. Carbon and nutrient inputs to the FBR are needed to sustain high rates of activity, but can also be responsible for microbial proliferation in the effluent water distribution pipeline, and loss injection well capacity.

The primary objective of this work was to fill critical knowledge gaps associated with the FBR that can be used to optimize the biological system for continuous operation and sustained performance.

The main findings are as follows:

1. **Iodine accumulation on FBR GAC.** Iodine is accumulating on the granulated activated carbon (GAC) in the 200W P&T FBR. Levels of total iodine measured were 857 µg/g GAC. Appreciable levels (160 µg/ mL) of I were also measured from stored sludge samples. Relatively low levels (6 µg/g) of Tc were associated with GAC.

2. **Dissimilatory nitrate reduction to ammonia.** In laboratory studies, FBR enrichment cultures were found to attenuate nitrate by a process called dissimilatory nitrate reduction to ammonia (DNRA). This result is important because DNRA does not eliminate nitrate from the system, but rather converts it to a biologically available form that can lead to microbial contamination in the effluent distribution pipelines and contribute to biofouling at the injection wells.

3. **Ferrocyanide impact on FBR activity.** Ferrocyanide was not inhibitory to the FBR enrichment cultures at concentrations below 50 µM (FeCN in Hanford groundwater is 0.5 µM), but exposure did slow growth in a concentration-dependent manner under aerobic and anaerobic conditions. Such a result could eventually negatively affect FBR activity and sustained performance.

These results contribute to a mechanistic understanding of the 200W P&T biological treatment system. With consistent sampling from the FBR and other locations within the 200 P&T Facility, functional relationships can be identified to optimize operations and support predicting system tolerances to new groundwater compositions. Results demonstrate that careful management of complex biological processes is needed to control biofouling at the 200W P&T injection wells.

[1] Thomle JN, BD Lee, GL Dai, and MJ Truex. 2017. *200 West Pump-and-Treat Facility Biofouling Assessment*. PNNL-26783, Pacific Northwest National Laboratory, Richland, WA.

Acknowledgments

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Acronyms and Abbreviations

200W P&T	200 West Pump-and-Treat
ASME	American Society of Mechanical Engineers
BSA	bovine serum albumin
CFR	Code of Federal Regulations
DNRA	dissimilatory nitrate reduction to ammonia
FBR	fluidized bed reactor
GAC	granulated activated carbon
ICP-MS	inductively coupled plasma mass spectroscopy
NQAP	Nuclear Quality Assurance Program
OD	optical density
P&T	pump and treat
PNNL	Pacific Northwest National Laboratory

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1.0 Introduction

Groundwater beneath the Central Plateau at Hanford contains a complex mixture of contaminants. Groundwater in the 200W Area of the Hanford Site is being treated *ex situ* by the 200W Pump-and-Treat (P&T) Facility, which removes key contaminants of concern (uranium, technetium, nitrate, carbon tetrachloride) from groundwater by a combination of chemical and physical processes, as well as a biological treatment for primary removal of nitrate. Treated water is then reinjected into the subsurface to contain and diminish groundwater contaminant plumes. However, the flow capacity of the injection wells decreases over time due to biofouling. This work focuses on the characterization of the 200 W P&T biological system. It is intended to provide new information that could be used to help guide decision making and optimization of the fluidized bed reactor (FBR) biological treatment system without compromising activity or performance of the facility.

As part of the 200W P&T Facility, the biological treatment system consists of twin FBRs that are operated under anoxic conditions to reduce nitrate, and provide additional capacity for the removal of carbon tetrachloride and hexavalent chromium from groundwater (Byrnes et al. 2013). The FBR contains granular activated carbon (GAC), which provides a substrate for bacterial colonization and biofilm formation, but can also provide a reactive surface(s) for inorganic contaminant adsorption. Prior to the work presented in this report, the surface composition of the GAC in the 200W P&T Facility had not been characterized sufficiently to identify GAC function(s).

As part of standard operations of the 200W P&T FBR biological treatment system, incoming groundwater is pH titrated with phosphoric acid and supplemented with a proprietary carbon source (MicroC® 4100) and micronutrient blend. Carbon and nutrient dosages are determined by a set of calculations that assume complete reduction of groundwater nitrate (Carlson et al. 2016; Lee and Lee 2016). Respiratory denitrification, defined as microbiological reduction of nitrate to gaseous products, is considered to be the primary pathway for groundwater nitrate removal in the 200W P&T Facility. Effluent water can contain excess carbon and nutrients, which can lead to microbial contamination throughout the effluent water distribution pipeline and contribute to the fouling of injection wells. The 200W P&T Facility injection wells were designed for 150 gpm effluent water, but regular cleaning (well re-habilitation and re-development) is required to maintain injection operations (see Thomle et al. 2017). Current trends suggests a consistent decline in the efficiency of the 200W P&T Facility injection wells.

Thomle et al. (2017) prepared a comprehensive engineering assessment of biofouling at the 200W P&T Facility and recommended operational changes and facility modifications to mitigate the consequences of biofouling. This investigation focused on identifying how the FBR is contributing to biofouling, and how modifying its operation can reduce biofouling at the injection wells. Since specific and diagnostic information is needed for reliable process optimization, this effort focused specifically on the conditions that contribute to or exacerbate biofouling at the 200W P&T Facility. The overarching goal is to fill critical knowledge gaps in the understanding of how the FBR biological treatment system functions and to more accurately define the biological requirements for continuous operation and sustained performance, while minimizing the factors that contribute directly to biofouling.

The objectives of this task were to (1) provide new information regarding the fate and distribution of iodine and technetium in the 200W P&T Facility, (2) provide specific information about nitrate attenuation and nutrient conditions, and (3) explore nitrate attenuation process tolerance to ferrocyanide concentrations, which may be toxic to microbial populations associated with the FBR.

2.0 Inorganic Contaminant Partitioning at 200W P&T

Hanford groundwater treated by the 200W P&T Facility contains, among other contaminants, radioiodine (I-129) and technetium (Tc-99). The RAD building of the P&T Facility is expected to remove the majority of Tc-99 in the groundwater by ion exchange chromatography. Aqueous iodine species have been assumed to be sequestered onto the GAC substrate in the FBR biological treatment system (Parker and Wellman 2017). Elemental analysis of the ion exchange resin, however, revealed significantly higher concentrations of I-127 (and Fe) than either I-129 or Tc-99 (Levitskaia et al. 2017). The fate of I-129 in the 200W P&T Facility is not fully known. The objective of this investigation was to analyze archived 200W P&T samples, specifically solids, filtrates, and concentrates, not only to demonstrate the retention of I-129 and Tc-99, but more specifically, to quantify the mass and distribution of both contaminants through the 200W P&T Facility.

2.1 Experimental Methods

GAC and concentrated solids (sludge, filtrate) from the 200W P&T Facility were retrieved from cold storage and analyzed for total Tc-99 and iodine (I). Sample locations within the facility are shown in Figure 1, and were collected on 04/02/2013, 08/04/2015, and 03/27/2017.

Briefly, nitric acid (8M) extractions were performed at a 1:5 solid to solution ratio according to method ASTM D5198. Samples were heated for 2 hours at 90°C, and filtered (0.45 μm) prior to analysis. Acid digestion combined with high temperature is known to volatilize iodine from the sample; therefore, a mathematical correction was applied to iodine values based on a previous analyses of pure standards and spiked controls. Analytical controls were performed in duplicate and included a sample blank (solutions only), a spiked blank (solution spiked with contaminant of interest), and a matrix spike. All filtrates were analyzed by inductively coupled plasma mass spectroscopy (ICP-MS) (Thermo Scientific X-Series II) for total technetium and iodine concentration.

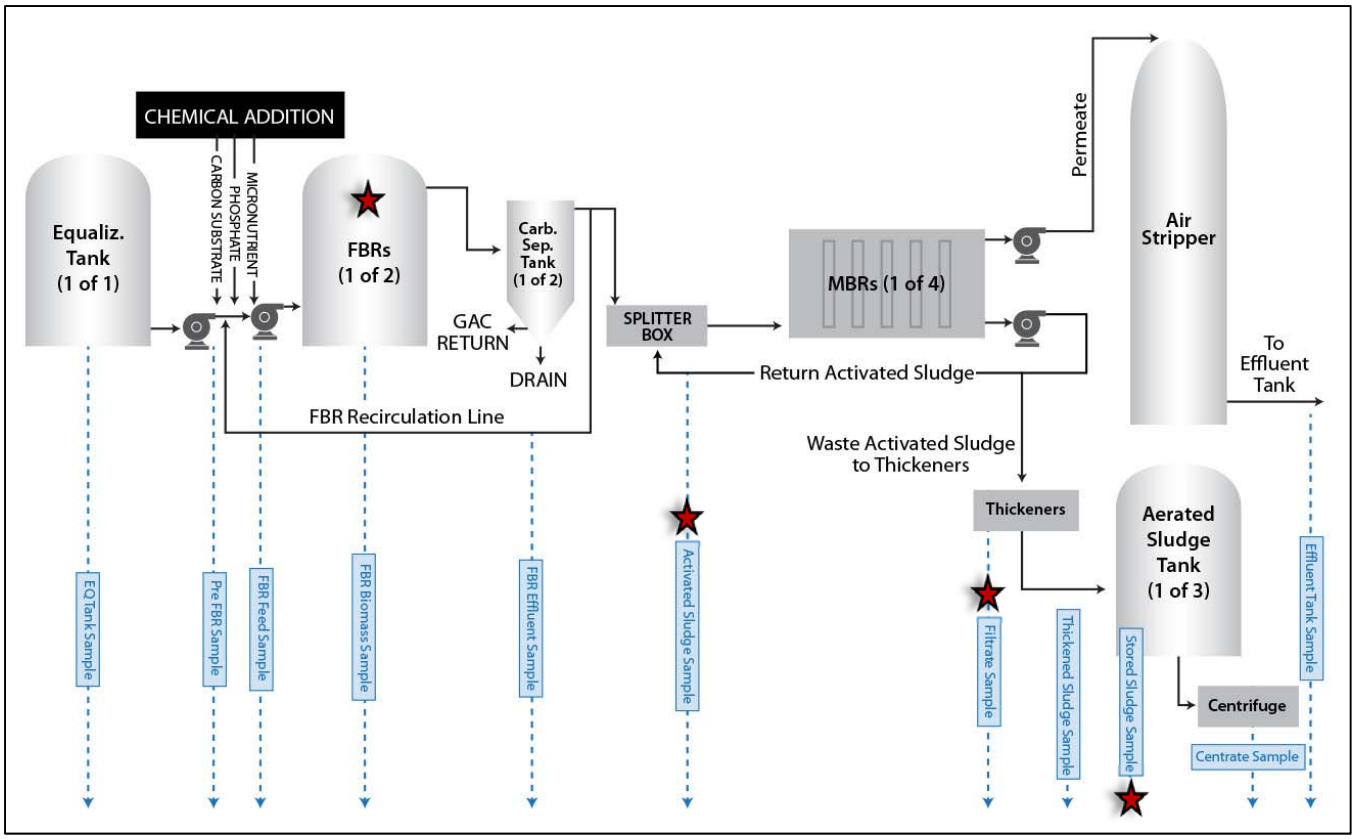

Figure 1. Diagram of the 200 West Pump-and-Treat Facility. Archived concentrate and filtrate samples collected throughout the plant (labeled by a red star) were submitted in duplicate for analysis of total technetium (Tc) and iodine (I) by ICP-MS.

2.2 Results

Iodine was detected from all of the GAC samples, one of the activated sludge samples (V16-Y52A; collected 08/04/2015), and both stored sludge samples (V10-Y71B; collected on 08/04/2015) (Table 1). Concentrations increased from 24.3-161.4 µg I / g GAC [corrected values 30.6-203.3 µg I / g GAC] (collected on 04/02/2013) to 680.1 µg of I / g GAC [corrected values, 857.0 µg I / g GAC] (collected on 03/27/2017) in approximately 4 years. Total iodine was detectable in a single activated sludge sample (V16-Y52A), though the concentration was low (6.4 µg I / g; corrected value of 8.0 µg I / g). Iodine concentrations in stored sludge samples (V10-Y71B) ranged from 120.5-130.6 ug I / g (corrected values, 151.8-164.0 µg I / g). Iodine was not detected from either of two filtrate samples (V-12-Y70A; collected on 08/04/2015).

Technetium was detected in all of the GAC samples but no other concentrate samples. Concentrations increased from 0.2-0.9 µg Tc / g GAC (collected on 04/02/2013) to 6.1 µg of Tc / g GAC (collected on 03/27/2017) in approximately 4 years. For comparison, Levitskaia et al. (2017) analyzed contaminant loading (3 yr) onto spent 200W P&T Purolite A530E IX resin and measured 58 µg I-127 / g resin, 0.06 µg I-129 / g resin, and 22 µg Tc-99 / g resin.

Table 1. Summary of Total Iodine (I) and Technetium (Tc) Concentrations from Samples at the 200W P&T Facility

ID	Sample Description	Location	Sampled Date	Units	Total I	Corrected Total I[(a)]	Total Tc
B2P0M9	GAC from FBR-A	Top of Bed	04/02/2013	µg / g	161.36	203.31	0.85
B2P0N1	GAC from FBR-A	Bottom of Bed	04/02/2013	µg / g	24.31	30.63	0.17
B39HP1	GAC from FBR-A	Top, Grab	03/27/2017	µg / g	680.17	857.01	6.05
B325P2-1	Activated Sludge	V16-Y52A	08/04/2015	µg / mL	ND	ND	ND
B325P2-2	Activated Sludge	V16-Y52A	08/04/2015	µg / mL	6.38	8.04	ND
B325K0-1	Filtrate	V-12-Y70A	08/04/2015	µg / mL	ND	ND	ND
B325K0-2	Filtrate	V-12-Y70A	08/04/2015	µg / mL	ND	ND	ND
B352P6-1	Stored Sludge	V10-Y71B	08/04/2015	µg / mL	120.50	151.83	ND
B352P6-2	Stored Sludge	V10-Y71B	08/04/2015	µg / mL			ND

(a) Corrected iodine concentrations take into account a 26% loss by I by volatilization during sample preparation.

2.3 Summary

Limited samples were available from the P&T facility for this investigation. Analysis of archived samples revealed that iodine is widely distributed throughout the 200W P&T Facility, and that technetium is not being fully captured by the IC resin in the RAD building.

The GAC in the 200W P&T FBR biological treatment system is retaining the most I and Tc relative to other facility samples that were available, and their concentrations are increasing over time. The mechanisms that determine binding capacity are still unknown. Identifying processes (adsorption, chemical, biological) that are involved in sequestering contaminants and quantifying their susceptibility for release will help to determine the GAC binding capacity across a range of future groundwater chemistries that may be routed to the 200W P&T Facility.

3.0 FBR Culture Characterization: Denitrification Activity

The 200W P&T FBR biological treatment system has been operating under the assumption that groundwater nitrate is being reduced and eliminated by a process of denitrification. Denitrification is a respiratory process where nitrate is the terminal electron acceptor that is sequentially reduced to nitrogenous gases (NO, N_2O, N_2). The process results in a net loss of N from the 200W P&T Facility. Denitrification is expected to be the primary nitrate attenuation pathway in the 200W P&T Facility because (1) the FBR biological treatment system was originally inoculated with a denitrifying seed culture, (2) analyses of grab samples taken from the FBR biological treatment system showed a high abundance of *nirS* and *nirK* functional genes that are specific to denitrifying bacteria, and (3) laboratory experimentation consistently demonstrated the rapid decrease in nitrate concentration following supplementation with a carbon source (Lee et al. 2014, 2015a,b,c, 2016, 2017; Morad et al. 2017). Once oxygen has been consumed from the FBR biological treatment system, denitrification is the highest energy yielding anaerobic respiratory process (Strohm et al. 2007), and thus should be the predominant mechanism for NO_3^- attenuation from groundwater.

The objective of this investigation was to identify nitrate attenuation mechanisms in the 200W P&T FBR biological treatment system and the potential impacts of groundwater chemistry on activity.

3.1 Experimental Methods

An FBR denitrifying enrichment culture was resuscitated from -80°C cold storage and maintained in synthetic groundwater. A series of experiments were conducted to quantitatively couple denitrification activity to groundwater composition and concentrations of micronutrients, iodine, and ferrocyanide. By providing accurate laboratory data that more narrowly define nitrate attenuation, the nutritional requirements for sustained activity and culture maintenance, the FBR can be optimized to potentially reduce biofouling at the injection wells.

Synthetic groundwater medium (Table 2) was prepared using the recipe detailed below using previously established procedures outlined by Truex et al. (2017).

Table 2. Synthetic Groundwater Medium

Constituent	Conc. (mg/L)	Mass for 1 L (g)
H_2SiO_3*nH_2O, silicic acid	15.3	0.0153
KCl, potassium chloride	8.20	0.0082
$MgCO_3$, magnesium carbonate	13.0	0.0130
NaCl, sodium chloride	15.0	0.0150
$CaSO_4$, calcium sulfate	67.0	0.0670
$CaCO_3$, calcium carbonate	150	0.1500

To mimic Hanford groundwater, the synthetic groundwater was composed of excess $CaCO_3$ (solubility is 14 mg/L) and stirred for 1 week to reach equilibrium. Prior to use, the groundwater was filtered by passage through a 0.2 µm pore size filter. The synthetic groundwater medium did not require pH adjustment (approximately 7.8), though pH was routinely recorded throughout experimentation.

Synthetic groundwater medium was supplemented with the carbon source (MicroC® 4100, 116 mL/L) and micronutrient blend (7.73 µL/L) used at the P&T Facility, and nitrate to be within range of influent groundwater concentrations (100 mg/L; 1.6 mM - final). Sodium lactate was also used as a defined, fermentable carbon source (1 mM final concentration). A stock FBR denitrifying enrichment culture was grown and propagated in fully composed synthetic groundwater medium until baseline denitrification activity was established for five consecutive medium exchange cycles. Once baseline activity was established with the stock culture, five independent experimental bioreactors were inoculated to measure nitrate attenuation in response to groundwater composition. Semi-continuous (batch) cultivation techniques were used for these experiments in an attempt to most closely mimic FBR operations while permitting the necessary experimental throughput.

Experimental bioreactors maintained a 1:50 aspect ratio for settled GAC to composed groundwater height. This ratio is much less than the actual FBR biological treatment system at the 200W P&T Facility; however, higher amounts of GAC could not be effectively mixed in the lab-scale reactors. Bioreactors were maintained in the dark at 25°C on a five-position magnetic stirrer to ensure efficient mixing of the groundwater and to keep the GAC fully suspended. All five bioreactors were identically prepared on fully composed synthetic groundwater and inoculated from a common stock culture.

Bioreactors were continuously operated until microbial biomass and denitrification activity stabilized, at which time experimental manipulations will be initiated. Composed synthetic groundwater (100 mL) was exchanged weekly to maintain a consistent concentration of carbon and nitrate. Individual bioreactor treatments were assigned to evaluate the effects of (1) trace nutrient composition, (2) iodine exposure (250 ppb), (3) ferrocyanide exposure (200 ppb), and (4) the combined effects of iodine and ferrocyanide (250 ppb and 200 ppb, respectively). The remaining bioreactor served as the no treatment control.

Aqueous sampling was performed daily for chemical analysis. Common anions were analyzed on a Dionex™ ICS-2000 ion chromatograph equipped with an IonPac™ AS185 column. Chromatography conditions maintained an eluent KOH concentration of 22 mM for 10 min, then increased to 40 mM for an additional 15 min. Prepared and purchased (SPEX CertiPrep) chemical standards were used for peak identification for the following compounds: lactate, acetate, nitrite, nitrate, phosphate, chloride, sulfate, and fluoride. Low range, Test ‘N Tube Direct Hach® kits were used to measure nitrate, ammonia, and total organic carbon per the manufacturer’s instructions. Microbial biomass was measured using the Pierce BCA Protein Assay kit following the manufacturer’s instructions (Thermo Scientific). Standard curves were prepared using bovine serum albumin (BSA), the dynamic range was determined to be between 25 and 2000 ug/mL ($R^2 = 0.99$).

Experiments were conducted with the 200W P&T nitrate attenuating enrichment culture that was originally obtained directly from the FBR biological treatment system and has since been maintained as a stable frozen stock in the laboratory. Preliminary growth studies were performed to ensure consistent results and performance with previous reports. Lab cultures were scaled up using MicroC® 4100 as the sole carbon source. MicroC® 4100 is a carbohydrate-based liquid feedstock formulated by Environmental Operating Solutions, Inc., for use in biological water and wastewater treatment plants. MicroC® 4100 is a custom formulation that is prepared for the 200W P&T Facility to support anaerobic nitrate-reduction by denitrifying bacteria. MicroC® 4100 did not produce consistent growth between bioreactors and biomass yields were low. As a complex carbon source, MicroC® 4100 is intended to support many diverse microbial and metabolic groups, thus establishing the development of a highly active microbial community in the FBR biological treatment system. In actuality, complex carbon sources can decrease operational control and reproducibility of complex biological systems (like the FBR) because it encourages the growth of metabolic competitors that unpredictably change the carbon substrate profile of the system. Heterotrophic and fermentative bacteria, for example, have been shown to strongly influence NO_3^- attenuation activity by quickly altering the quantity and quality of carbon supplies available for nitrate reducing bacteria (van den Berg et al. 2016, 2017a,b). Poor growth performance of GAC samples on MicroC® 4100 has been shown prior (Morad et al. 2017). Subsequent experiments adopted lactate as the sole carbon/energy source.

3.2 Results

Dense biomass was quickly established for the 200W P&T denitrifying enrichment culture in five independent bioreactors containing AGW medium with lactate as the sole carbon/energy source. Iodate and ferrocyanide treatments (250 and 200 ppb, respectively) had no discernable effects on cell density or nitrate attenuation activity during repeated oxic-anoxic growth cycles. Representative IC results from nitrate attenuation experiments are summarized in Table 3. Available nitrate (5-10 mM) was quickly consumed (8-16 hours) following the onset of anaerobic conditions (N_2 sparging). As lactate levels decreased, peaks for a number of co-eluting products (2-3) steadily increased. Co-elution of acetate and formate reference standards with lactate was validated by IC; suggesting possible lactate fermentation to a mixture of short chain fatty acids. By 8 hours of anaerobic growth on nitrate containing artificial groundwater, a consistent increase in pH coincided with the accumulation of ammonia. Eventually, alkaline conditions became inhibitory (~pH 10), resulting in the accumulation of OC (fermentation products) and NO_2^-. Activity could be fully restored by air sparging, whereby the ammonia was utilized for growth, or partially restored by medium exchange (i.e., ammonia dilution). This result has not been previously reported because all prior experimentation with the FBR enrichment culture was conducted in buffered growth medium and only nitrate depletion was measured, not the conversion to reduced nitrogenous products. These experiments show that nitrate attenuation by the 200W P&T FBR enrichment culture includes a respiratory process called dissimilatory nitrate reduction to ammonia (DNRA), not solely denitrification as previously assumed.

It is important to acknowledge that the nitrogen mass conversions presented in Table 3 do not balance. Not all of the ammonia produced will accumulate in the system. Some fraction was assimilated, as indicated by the sharp increase in microbial biomass measured as total protein. Moreover, nitrite was detected in all of the bioreactors, though inefficient peak resolution did not permit quantitation. Finally, because GAC was incorporated into these experiments solid phase chemistry measurements should be considered. We only measured water chemistry in these experiments.

Although the composition of the 200W P&T FBR biological treatment system may have changed relative to the enrichment culture, the enrichment culture is the most reliable and accurate representation of the FBR microbial community available. These findings are also corroborated by previous lines of inquiry, analyses, and the most current effluent water chemistry data from the 200W P&T Facility. Therefore, this analysis is presumed to be pertinent to the current state and operation of the biological treatment system.

Table 3. Nitrate Attenuation by the 200W P&T enrichment culture. Groundwater treatments included iodate (250 ppb) and/or ferrocyanide (200 ppb). Outcomes are presented as the measured change (delta, Δ) in values over the course of a 64 hour experiment.

$\Delta_{(t=64\ hr)}$

Bioreactor	Treatment	pH (U)	Protein (μg/mL)	NO_3^- (mg/L)	NO_3^- (mg/L)	NH_3 (mg/L)
1	Control	+1.5	+120.0	-3.94	+0.37	+2.12
3	IO_3	$^+$2.0	+292.9	-2.93	+0.35	+1.59
4	$Fe(CN)_6^{4-}$	$^+$2.0	+246.4	-3.08	+0.42	+1.04
5	IO_3^-/ $Fe(CN)_6^{4-}$	$^+$2.0	+127.1	-3.38	+0.31	+1.59

Experiments were conducted at 5 ppm NO_3^-. Nitrite (NO_2^-) was detected for all bioreactors though the peak co-eluted and could not be reliably quantified.

3.3 Summary

Lee et al. (2017) and Thomle et al. (2017) described changes in groundwater chemistry as it moves through the 200W P&T Facility. Ammonia levels in untreated (native) groundwater are typically very low, around 8 µg /L, and ammonia is not consistently measured during routine monitoring at the 200W P&T Facility. Ammonia levels do, however, spike in the FBR biological treatment system and concentrations decrease as effluent water moves through the facility. Biologically significant levels of ammonia have been measured in effluent water collected from transfer buildings and injection wells. Any available ammonia in effluent water will be aggressively utilized for microbial growth and activity. Thus, sources of ammonia can contribute to well fouling and need to be minimized to control biofouling of the effluent distribution pipeline and injection wells.

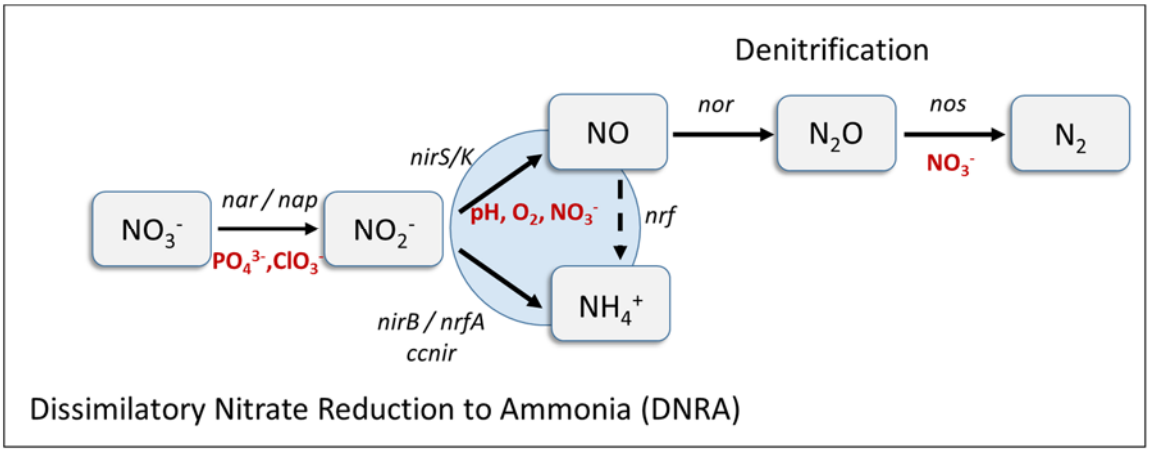

Figure 2. Competing Anaerobic Nitrate Reduction Pathways. The enzymes that catalyze specific reactions are listed in italics. Relevant environmental regulators of key reactions are in red and the pathway bifurcation (blue halo) is tightly controlled by a number of enzymatic and metabolic regulators.

Dissimilatory nitrate reduction to ammonia (DRNA), or ammonification, describes one pathway for the anaerobic reduction of nitrate. DNRA and denitrification (reduction of nitrate to nitrogenous gases) are distinct metabolic processes that compete for nitrate in the environment (Figure 2). The results reported here are relevant to the FBR biological treatment system because unlike denitrification, which results in the net loss of nitrogen, DNRA retains nitrogen in the system by converting it to a highly accessible (bioavailable) form, as soluble ammonia. DNRA in the FBR biological treatment system could be an important and continual source of ammonia at the 200W P&T Facility, contributing to biofouling of injection wells.

Denitrification is a more energetically favorable reaction than DNRA but the enzymatic pathway is energetically expensive, giving a competitive advantage for DNRA bacteria. Strohm et al. (2007) illustrated this point through a series of growth studies on simple substrates that showed that DNRA produced 2x higher cell mass per mol of NO_3^- than denitrification. Denitrifying bacteria typically have a high affinity for organic carbon substrates, but DNRA bacteria have a higher affinity for NO_3^-. The published literature has clearly established the high frequency with which denitrifying activity (communities) in natural and engineered systems are overcome and replaced by DNRA activity (communities) over time (Kessler et al. 2018; Rivett et al. 2008; Tiedje et al. 1982; van den Berg et al. 2017ab), particularly in conditions when the organic carbon / nitrate ratio is high (Giblin et al. 2013; Strohm et al. 2007). The likelihood that DNRA is

the predominant nitrate attenuation pathway occurring in the FBR biological treatment system and is providing a consistent supply of ammonia in the 200W P&T Facility would need to be confirmed using reliable and accurate molecular tools and additional effluent testing.

Environmental conditions that tend to favor DNRA over denitrification include a consistent supply of OC, carbon quality (fermentable substrates), low concentrations of nitrate, high C/N ratio, high temperatures, and variable pH, nitrite, and sulfide concentrations. Moreover, DNRA populations (and activity) are generally much less sensitive to changing environmental conditions than denitrifying bacteria (Rivett et al. 2008; van den Berg et al. 2016). It is improbable to assume that all DNRA activity can be eliminated, but it is conceivable that conditions in the FBR biological treatment system could be effectively managed to better control nitrate attenuation pathways in favor of denitrification to prevent unwanted production of ammonia.

4.0 FBR Culture Sensitivity to Cyanide

During the 1950s, ferrocyanide containing waste generated by the U Recovery Plant was discharged to the BY cribs and to a trench in the northern part of the 200 East Area (Hartman and Dresel 1998). Resulting groundwater now contains significant levels of cyanide, with other co-located contaminants (e.g., Tc-99 and nitrate).

200 East Area groundwater is being considered for processing at the 200W P&T Facility; however, cyanide is a potent toxin and has the potential to interfere with biological treatment processes at the 200W P&T Facility. These processes are principally responsible for removing nitrate from the groundwater.

The objective of this investigation was to provide a preliminary investigation into the inhibitory effects of ferrocyanide on the growth and activity of the FBR denitrifying enrichment culture. Aerobic and anaerobic experiments were performed to assess toxicity, and to discern the potential ferrocyanide interactions.

4.1 Experimental Methods

The 200W P&T FBR denitrifying enrichment culture (described by Szecsody et al. 2017) was resuscitated from -80°C cold storage and maintained on synthetic groundwater medium for aerobic and anaerobic growth experiments. The base synthetic groundwater medium was prepared following the recipe described elsewhere in this report (Truex et al. 2017). For growth experiments, synthetic groundwater medium was supplemented with lactate (sodium lactate, 1 mM final concentration), phosphate (KH_2PO_4, 100 µM final concentration), and nitrate ($NaNO_3$, 1.6 mM, final concentration) to be within range of influent groundwater concentrations to the 200W P&T Facility. Fully composed synthetic groundwater medium was pH 7.8.

Aerobic growth studies were performed in sterile culture tubes with plastic snap-caps. Anaerobic growth studies were performed in glass serum bottles with butyl rubber septa and crimp seals. Serum bottles were flushed with sterile N_2 for 5 minutes to decrease O_2 levels prior to inoculation. All experiments were performed in triplicate at 25°C. A filter sterilized (0.5 mM) ferrocyanide stock solution was diluted to establish an experimental concentration range of 0 to 200 µM. Ferrocyanide additions did not affect the final pH of the composed synthetic groundwater medium. Bacterial biomass and ferrocyanide was determined spectrophotometrically by measuring optical density at 610 nm, and absorbance at 320 nm, respectively, using the Eon™ microplate spectrophotometer (BioTek).

4.2 Results

The results for the aerobic dose-response experiments are shown in Figure 3. The growth responses between the no ferrocyanide positive control cultures and cultures exposed to the lowest concentration of ferrocyanide (1µM) were fairly consistent; however, higher concentrations of ferrocyanide (>10 µM) exhibited a long lag phase before the onset of growth. In fact, no growth was measured for any of these treatments for the first 3 days of incubation. Following this extended lag phase, the growth rate and biomass yield for the all cultures between 1 and 50 µM ferrocyanide were consistent with the no ferrocyanide positive control cultures. Growth was severely impeded by ferrocyanide at the highest concentrations tested, 100 and 200 µM (graphs are overlapping).

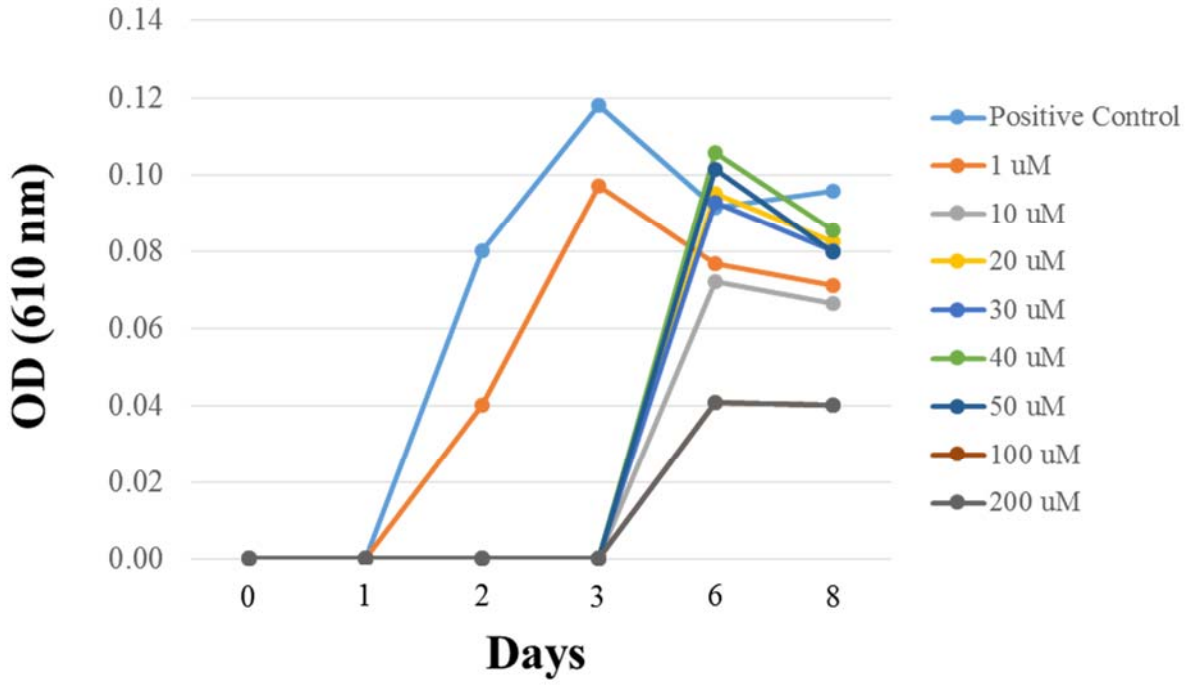

Figure 3. Aerobic Growth of the FBR Enrichment Culture at Increasing Concentrations of Ferrocyanide. Growth experiments were conducted in triplicate. Standard deviation was < 10%.

The results for the anaerobic dose-response experiments are shown in Figure 4. The growth responses between the no ferrocyanide positive control cultures and cultures exposed to the lower concentrations of ferrocyanide (1 to 50 µM) were fairly consistent. The growth rate of the ferrocyanide treated cultures was negatively impacted, but growth yields were consistent and generally greater than those produced under aerobic conditions. The highest concentrations of ferrocyanide (100 and 200 µM) became inhibitory.

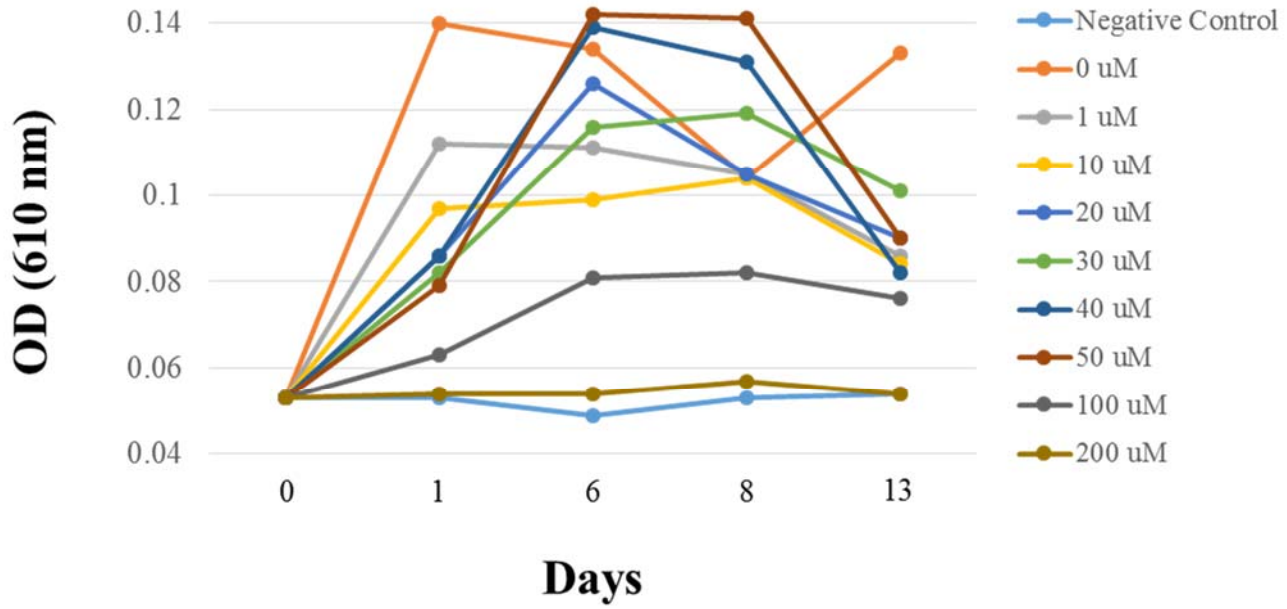

Figure 4. Anaerobic Growth of the FBR Enrichment Culture at Increasing Concentrations of Ferrocyanide. Growth experiments were conducted in triplicate. Standard deviation was < 10%.

Ferrocyanide concentrations were measured spectrophotometrically at the end of the anaerobic growth experiments (Figure 5). The analysis was limited to the concentration range from 1 to 50 µM because these levels were the most relevant, and higher concentrations were inhibitory. The absorbance spectra between filter sterilized spent medium (0.2 µm to remove biomass) and un-inoculated controls were not statistically different (t-test; $P < 0.05$). These results indicate that ferrocyanide was not degraded during anaerobic growth of the FBR denitrifying enrichment culture.

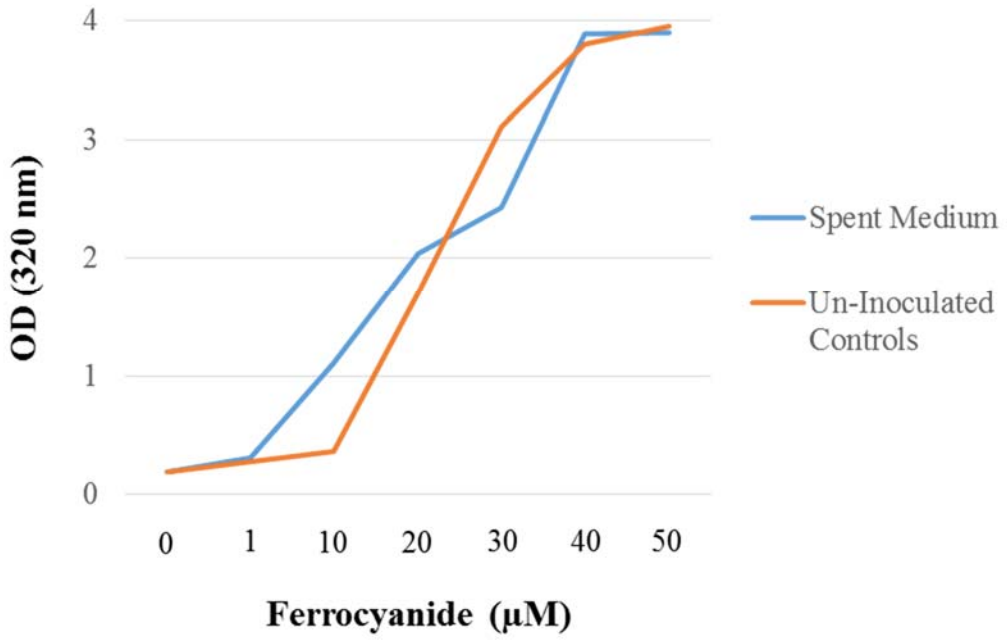

Figure 5. Ferrocyanide Measurements Pre- and Post-Anaerobic Growth of the FBR Enrichment Culture

4.3 Summary

Iron complexes with cyanide are extremely stable and presumed to exhibit low toxicity to biological systems and high recalcitrance in environments above pH 2 (Dzombak et al. 2006; Gensemer et al. 2006). It is not clear, however, if the ferrocyanide complex could dissociate to form free CN- over long residence times in Hanford groundwater.

A variety of bacteria and fungi can degrade ferri- and ferro-cyanide, particularly at more alkaline conditions (Dursun and Aksu 2000; Luque-Almagro et al. 2005; Morris et al. 2005). While CN^- is primarily inhibitory to aerobic metabolic processes, there are reports of CN^- inhibition of anaerobic microbial processes, such as denitrification (Kapoor et al. 2016; Kim et al. 2011). The short-term growth experiments performed here, while preliminary, suggest the ferrocyanide complex was stable during short term microbial growth studies. The highest concentrations of ferrocyanide tested (100 and 200 µM) did inhibit culture growth, but the mode of action in these experiments was not specifically investigated.

Aerobic and anaerobic growth responses to increasing concentrations of ferrocyanide were not consistent with the expected response for chemical toxicity of free cyanide. The extended lag phase during aerobic growth conditions slightly improved biomass yields under anaerobic growth conditions, and slower growth rates implies that ferrocyanide may be reacting chemically, possibly with O_2. While preliminary, it is clear that ferrocyanide does affect the growth of the 200W P&T FBR denitrifying enrichment culture, but more work is needed to resolve the biological mode of action under different O_2 regimes.

5.0 Quality Assurance

The results presented in this report originate from work governed by the Pacific Northwest National Laboratory (PNNL) Nuclear Quality Assurance Program (NQAP). The NQAP implements the requirements of the United States Department of Energy Order 414.1D, *Quality Assurance*, and 10 CFR 830 Subpart A, *Quality Assurance Requirements*. The NQAP uses ASME NQA-1-2012, *Quality Assurance Requirements for Nuclear Facility Applications* as its consensus standard and NQA-1-2012 Subpart 4.2.1 as the basis for its graded approach to quality.

Two quality grading levels are defined by the NQAP:

Basic Research - The required degree of formality and level of work control is limited. However, sufficient documentation is retained to allow the research to be performed again without recourse to the original researcher(s). The documentation is also reviewed by a technically competent individual other than the originator.

Not Basic Research - The level of work control is greater than basic research. Approved plans and procedures govern the research, software is qualified, calculations are documented and reviewed, externally sourced data is evaluated, and measuring instrumentation is calibrated. Sufficient documentation is retained to allow the research to be performed again without recourse to the original researcher(s). The documentation is also reviewed by a technically competent individual other than the originator.

The work supporting the results presented in this report was performed in accordance with the *Not Basic Research* grading level controls.

6.0 Conclusions

Biofouling is a reoccurring issue for 200W P&T injection wells. This report provides information that can be used to evaluate biological component aspects related to P&T system performance, biofouling, and in anticipation of future influent streams. Efficient biological treatment of groundwater and process optimization requires a thorough understanding of the fundamental processes involved in contaminant attenuation, as well as the process vulnerabilities to groundwater perturbation. Laboratory analyses of facility samples and experiments can provide valuable support to P&T operations and process optimization.

The main findings are summarized below:

1. **Iodine and low levels of technetium are accumulating on the GAC in the FBR.** Results showed temporal accumulation of I and Tc-99on the GAC in the FBR. Iodine concentrations were much higher than Tc-99, and iodine was also detected in stored sludge samples. The mechanisms involved in sequestering iodine onto the GAC are not known but would be important to define in order to approximate the binding capacity of I onto the GAC, and to predict the stability of I-GAC complexes against different groundwater chemistries that may be considered for future treatment at 200W P&T.

2. **Mechanisms of nitrate attenuation in the FBR should be specifically evaluated.** In the laboratory tests, FBR enrichment cultures reduced nitrate by a process called dissimilatory nitrate reduction to ammonia (DNRA). This result is important because DNRA does not eliminate nitrate from the system, but rather converts it to a biologically available form that can lead to microbial contamination in the effluent distribution pipelines and contribute to biofouling at the injection wells. Diagnostic molecular tools are available to differentiate and monitor nitrate attenuation processes from FBR samples,

specifically DNRA. Molecular biological tools could be used more effectively in combination with routine water quality measurements to provide a more robust measure of facility performance and to isolate sources of microbial contamination in the effluent distribution system.

3. **Ferrocyanide appears to be chemically reactive but short-term experiments suggest biological impacts may be minor.** At typical groundwater concentrations, ferrocyanide was not inhibitory to the FBR enrichment cultures but exposure did retard aerobic and anaerobic growth in a concentration dependent manner. Follow-up studies should consider longer term interactions to specifically evaluate the potential for ferrocyanide sequestration on GAC (preferably used GAC from the FBR), the impacts on nitrate attenuation in an established microbial system (i.e., the extant community in the FBR, and to define the chemical reactivity of ferrocyanide (potentially for the production of reactive oxygen species). All of these interactions could have important implications for the performance and sustained activity of the FBR, but may also influence the stability of I and other elements that are accumulating on the GAC in the FBR.

7.0 References

10 CFR 830, Subpart A. 2011. "Quality Assurance Requirements." Code of Federal Regulations, U.S. Department of Energy, Washington, D.C.

ASME NQA-1-2012. *Quality Assurance Requirements for Nuclear Facility Applications*. The American Society of Mechanical Engineers, New York, New York.

Byrnes ME, S Simmons, and J Morse. 2013. "Startup of the New 200 West Pump-and-Treat, Hanford Site, Richland, Washington." Waste Management Conference, Phoenix, AZ.

Carlson M, S Simmons, B Barrett, J Morse, M Byrnes, and E Laija. 2016. "200 West Pump and Treat: The First Three Years." WM2016 Conference, March 6-10, Phoenix, AZ.

DOE Order 414.1D. 2011. *Quality Assurance*. U.S. Department of Energy, Washington, D.C. Approved 4/25/2011.

Dursun AY and Z Aksu. 2000. "Biodegradation kinetics of ferrous(II) cyanide complex ions by immobilized *Pseudomonas fluorescens* in a packed bed column reactor." *Process. Biochem.* 35:615-622.

Dzombak D, R Ghosh, and T Young. 2006. "Physical-Chemical Properties and Reactivity of Cyanide in Water and Soil." In *Cyanide in Water and Soil: Chemistry, Risk, and Management.* Dzombak D, R Ghosh, and T Young, T (eds.). Taylor & Francis, Boca Raton, Florida, pp. 57-92.

Gensemer RW, DK DeForest, AJ Stenhouse, CJ Higgins, and RD Cardwell, R.D. Aquatic toxicity of cyanide. In *Cyanide in Water and Soil: Chemistry, Risk, and Management*, Dzombak, D., Ghosh, R.; Young, T., Eds. Taylor & Francis, Boca Raton, FL, 2006. pp. 251-284.

Giblin AE, CR Tobias, B Song, N Weston, GT Banta, and VH Rivera-Monroy. 2013. "The importance of dissimilatory nitrate reduction to ammonium (DNRA) in the nitrogen cycle of coastal ecosystems." *Oceanography* 26(3):124–131.

Hartman MJ, PE Dresel, et al. 1998. *1998 Hanford Site Groundwater Monitoring for Fiscal Year 1997*. PNNL-11793, Pacific Northwest National Laboratory, Richland, WA.

Kapoor V, M Elk, X Li, and JW Santo Domingo. 2016. "Inhibitory effect of cyanide on wastewater nitrification determined using SOUR and RNA-based gene-specific assays." *Letters in Applied Microbiology* 63:155-161. doi:10.1111/lam.12603

Kessler A. J., Roberts, K. L., Bissett, A., & Cook, P. L. M. (2018). "Biogeochemical controls on the relative importance of denitrification and dissimilatory nitrate reduction to ammonium in estuaries." Global Biogeochemical Cycles 32:1045–1057.

Kim YM, HU Cho, DS Lee, D Park, and JM Park. 2011. "Comparative study of free cyanide inhibition on nitrification and denitrification in batch and continuous flow systems." *Desalination* 279:439-444. doi:10.1016/j.desal.2011.06.026

Lee BD, EE Eisenhauer, DL Saunders, SD Hoyle, E Cordova, SA Simmons, MA Carlson, JG Morse, and MH Lee. 2016. *Dominance of nitrite reductase genes in a fluidized bed biofilm reactor treating nitrate contaminated groundwater at the Hanford Site*. PNNL-SA-119268, Pacific Northwest National Laboratory, Richland, WA.

Lee BD, S Saurey, EA Cordova, M Carlson, D Saunders, S Brooks, W Garcia, B Christiansen, MH Lee. 2017. *Chemical and microbial sampling of the Hanford 200 west pump and treat biological plant*. [Unpublished]

Lee BD, SD Saurey, EA Cordova, SA Simmons, JG Morse, EE Eisenhauer, and MH Lee. 2015a. "Dominance of nitrite reductase genes in a fluidized bed biofilm reactor treating nitrate contaminated groundwater at the Hanford Site." PNNL-SA-113585, Pacific Northwest National Laboratory, Richland, WA.

Lee BD, MA Carlson, A Menniti, K Martins, J Boltz, SA Simmons, JG Morse, ME Byrnes, SD Saurey, EA Cordova, EER Eisenhauer, and MH Lee. 2015b. *Microbial shifts and related performance of a fluidized bed bioreactor operated as part of the 200 West Groundwater Treatment Facility at the Hanford Site*." PNNL-SA-107626, Pacific Northwest National Laboratory, Richland, WA.

Lee BD, MA Carlson, A Menniti, K Martins, J Boltz, SA Simmons, JG Morse, ME Byrnes, SD Saurey, EA Cordova, EE Eisenhauer, and MH Lee. 2015c. *Optimization-based monitoring of a fluidized bed biofilm reactor operated as part of the 200 West Area Pump and Treat Facility at the Hanford Site*. PNNL-SA-113590, Pacific Northwest National Laboratory, Richland, WA.

Lee BD, SD Saurey, EA Cordova, SA Simmons, JG Morse, EER Eisenhauer, and MH Lee. 2014. *Functional characterization of the microbial community in a fluidized bed bioreactor treating nitrate contaminated groundwater at the Hanford Site*. PNNL-SA-105720, Pacific Northwest National Laboratory, Richland, WA.

Lee MH and BD Lee. 2016. *Letter Report: Optimization and mitigation of environmental impacts from effluent discharge activities associated with the 200W P&T System*. PNNL-25925, Pacific Northwest National Laboratory, Richland, WA.

Levitskaia TG, EL Campbell, SD Chatterjee, GB Hall. 2017. *Analysis of technetium ion exchange resin from the 200 West Pump-and-Treat Facility*. PNNL-26933, Pacific Northwest National Laboratory, Richland, WA.

Luque-Almagro VM, M-J Huertas, M Martínez-Luque, C Moreno-Vivián, MD Roldán, LJ García-Gil, F Castillo, and R Blasco. 2005. "Bacterial degradation of cyanide and its metal complexes under alkaline conditions. *Appl. Environ. Microbiol.* 71:940-947.

Morad J, EA Cordova, D Saunders, BD Lee. 2017. *Iodate transformation in a denitrifying biofilm taken from the Hanford Site 200 west pump and treat system.* [Unpublished]

Morris K, H Zhao, and R John. 2005. "Ferricyanide-mediated microbial reactions for environmental monitoring." *Aust. J. Chem*. 58:237-245

NQAP-2012, *Nuclear Quality Assurance Program (NQAP) Manual*. Pacific Northwest National Laboratory, Richland, WA.

Parker KE and DM Wellman. 2017. *Design and testing of a novel system using supercritical water to remove iodine from activated carbon*. PNNL-26826, Pacific Northwest National Laboratory, Richland, WA.

Rivett MO, SR Buss, P Morgan, JWN Smith, and CD Bemment. 2008. "Nitrate attenuation in groundwater: A review of biogeochemical controlling processes." *Wat. Res.* 42:4215-4232.

Strohm TO, B Griffin, WG Zumft, and B Schink. 2007. "Growth yields in bacterial denitrification and nitrate ammonification." *Appl. Environ. Microbiol.* 73:1420-1424

Thomle JN, BD Lee, GL Dai, and MJ Truex. 2017. *200 West Pump-and-Treat Facility Biofouling Assessment*. PNNL-26783, Pacific Northwest National Laboratory, Richland, WA.

Tiedje JM, AJ Sexstone, DD Myrold, and JA Robinson. 1982. "Denitrification: ecological niches, competition, and survival." *Antonie van Leeuwenhoek* 48:569-83.

Truex MJ, JE Szecsody, N Qafoku et al. 2017. *Contaminant attenuation and transport characterization of 200-DV-1 Operable Unit Sediment Samples*. PNNL-26208; RPT-DVZ-AFRI-037 830403000, Pacific Northwest National Laboratory, Richland WA.

Van den Berg EM, JL Rombouts, JG Kuenen, R Kleerebezem, and MCM van Loosdrecht. 2017a. "Role of nitrite in the competition between denitrification and DNRA in a chemostat enrichment culture." *AMB Expr 7:91.*

Van den Berg EM, MP Elisario, JG Kuenen, R Kleerebezem, and MCM van Loosdrecht. 2017b. "Fermentative bacteria influence the competition between denitrifiers and DNRA bacteria." *Front. Microbiol.* 8:1684.

Van den Berg EM, M Boleij, JG Kuenen, R Kleerebezem, and MCM van Loosdrecht. 2016. "DNRA and denitrification coexist over a broad range of acetate/N-NO3- ratios, in a chemostat enrichment culture." *Front. Microbiol.* 7:1842

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